REMARKS

Objections to the claims:

Claim 35 has been objected to because the phrase "around the limb" is considered by the examiner to be ambiguous. Applicants have amended the claim to delete the phrase as requested by the examiner.

Claims 1 and 39 have been objected to because it is the examiner's opinion that step (a) does not clearly parallel the preamble of the claim. Applicants have amended the claims as requested by the examiner.

Claim 39 has been objected to because the examiner feels that step (b) is too wordy. Applicants have amended the claim as requested by the examiner.

Claim 39 has been objected to because the phrase "and expressing the polynucleotide" and "wherein inserting..." are unclear. Applicants have amended the claim to obviate the rejection.

Rejection of the claims under 35 USC 112:

Claims 1 and 39 have been rejected under 35 U.S.C. 112, first and second paragraph. The examiner contends that no explicit support can be found in the specification for the term "non-invasive". The action states "The specification does not implicitly support applying pressure without inserting an instrument of device into the body through the skin." The action further provides a definition of invasive obtained from Steadman's Medical Dictionary. This definition corresponds with the description provided by the Applicants' specification (page 5 lines 13-24). Applicants have not claimed a non-invasive injection device. Applicants further acknowledge that some of their examples include the much more invasive use of clamps applied directly to limb vessels. Comparing example 10 (starting on page 32) with example 8 (starting on page 31) demonstrates that using a cuff can substitute for invasively clamping individual vessels in occluding blood flow. The use of the non-invasive cuff is a significant improvement over using individual clamps because it makes the method much less invasive, faster and easier to perform, and provides improved occlusion of blood flow to and from the limb.

The claims have been rejected under 35 U.S.C. 112, second paragraph, for not reasonably provided enablement for injecting the polynucleotide into the limb proximally to the applied pressure and obtaining delivery of polynucleotides to skeletal muscle cells of the limb distal to the applied pressure. Applicants have amended claims 1 and 39 to specify that the polynucleotides are injected distal to the applied pressure. Support for the amendment can be found in the specification on page 23 lines 22-23, page 32 lines 11-13. The specification states that the occlusion (cuff) is placed proximal to the injection site. This is equivalent to injecting the solution distal to the applied pressure.

The claims have been rejected under 35 U.S.C. 112, second paragraph, for not reasonably provided enablement for expressing a polynucleotide in a skeletal muscle cell by injecting a viral vector. Applicants respectfully disagree. Applicants have supplied with the amendment filed May 9, 2003 a declaration under 37 CFR 1.132 providing evidence that the described invention is enabling for expressing a polynucleotide in skeletal muscle cells by injecting a viral vector. The delivery examples provided in the declaration are done using clamps applied to individual blood vessels to occlude blood flow rather than a cuff applied around the limb. However, comparing examples 10 (starting on page 32) with example 8 (starting on page 31) demonstrates that using a cuff can substitute for invasively clamping individual vessels in occluding blood flow. Therefore, it can reasonably be expected that viral vectors would be delivered if a cuff had been used in the example provided in the 1.132 declaration.. That the delivery of viral vectors was contemplated in the specification as originally filed is provided in the specification on page 15 lines 13-25.

The examiner further states that the example provided in the 1.132 declaration does not correlate with the specification disclosure because it is not readily apparent that applicants considered using both papaverine and collagenase. Page 5 lines 26-28 clearly teaches that the use of pharmaceutical or biologically-active agents to increase vascular permeability is contemplated. Compounds that increase permeability of the vessel by causing a change in cells within the vessel wall (such as papaverine), biologically-active molecules that interact with specific receptors within vascular cells (such as VEGF) and enzymes that digest extracellular material are all explicitly contemplated (paragraph bridging pages 16-17). Collagenase is an enzyme that digests extracellular connective material and therefore was inherently contemplated. The use of agents to increase vascular permeability should not be limited to only one agent.

Finally, the examiner states that the use of $5x10^8$ adenoviral particles is not taught in the specification and that the volumes taught in the specification are limited to non-viral vectors. The volumes listed for delivering non-viral vectors are enabling for naked DNA, non-viral DNA complexes and viral vectors as evidenced the specific contemplation of naked DNA, non-viral DNA complexes and viral vectors (page 3 lines 1-6 and page 15 lines 21-25), the examples provided in the specification and the examples provided in the declaration. The explicit number of viral particles to inject will be dependent on the amount of tissue to which the vector is to be delivered and can be readily determined by those practicing the art without undo experimentation. One need simply do routine experimentation to optimize the number of viral particles to include in the injection solution.

The claims have been rejected because the specification does not provide adequate guidance for administering an immunosuppressive agent. Applicants respectfully disagree. On page 2 line 10-12, Applicants point out that any protein expressed from a delivered gene may "be degraded into peptides, which may be presented to the immune system." That transgene expression can be limited by host immune response was well known to those skilled in the art at the time the application was filed is evidence by Potter MA et al. Annals of the New York Academy of Sciences, 1999 Vol. 875 pp.159-74; Michou A et al Gene Therapy, 1997 Vol. 4 pp. 473-482; Tripathy SK et al. Nature Medicine, 1996 Vol. 2 No. 5 pp. 545-550 (see abstract); and Wilkinson et al. British Medical Bulletin, 1995 Vol. 51 No. 1 pp. 205-216 (see abstract). Example 5, starting on page 28 demonstrates that immunosuppression of the mammal provides for increased expression of the delivered transgene for a longer period of time. Therefore, it is the Applicants' opinion that given the knowledge in the art at the time of filing, the Applicants' specification provides adequate guidance for practicing the invention.

The claims have been rejected under 35 U.S.C. 112, second paragraph, for not reasonably provided enablement for delivering any polynucleotide as broadly claimed. Applicants have amended claim 1 to cite a polynucleotide encoding a protein operably linked to a promoter to obviate the rejection. Applicants have amended claim 39 to cite a polynucleotide encoding an expressible sequence operably linked to a promoter. Support for the amendments can be found in the specification on page 1 lines 20-24, page 6 lines 18-24, and page 6 line 28 to page 7 line 4.

The examiner states that the limitation of the procedure not diminishing use of the limb is unclear because it is uncertain if the limitation refers to function of the limb or frequency of use of the limb. These two parameters are not distinct. It is reasonable that if function is diminished in a limb the mammal would naturally use the limb less frequently. Page 3 lines 16-17 state that "it is important that the full function of the mammal's limbs subsequent to delivery is maintained". Full function inherently suggests both function and frequency of use. Page 22 lines 15-20 state that blood flow is impeded for substantially less time than would cause ischemic tissue damage and that no histological evidence of ischemic muscle damage has been observed in mammals following the procedure. Page 22 lines 26-29 state that only minimal and inconsequential intimal changes in the arteries were observed. Page 23 lines 4-5 state that no toxic effects were observed in rodents or monkeys. Page 25 lines 17-20 state that all monkeys tolerated the procedure well, had full function of their limbs following the procedure and no nerve damage was apparent. Page 25, lines 22-24 states that monkeys were not in any discomfort beyond that of normal surgical recovery. Decrease frequency of limb use following the procedure would not constitute "no discomfort beyond that of normal surgical recovery." Page 28 lines 4-22 state that no elevation or only transient elevation was observed for a number of enzymes and that the vast majority of muscle tissue did not show any sign of pathology.

Rejection of the claims under 35 USC 102:

Claim 39 has been rejected under 35 U.S.C. 102(e) as being anticipated by Draijer-van der Kaaden (US Patent 6,495,131). The examiner states that the method of '131 impedes inflow and outflow of blood through the limb and that claim 39 is not limited to impeding all blood flow out of the limb. Applicants respectfully disagree. Step (a) [formerly step (b)] recites "applying pressure non-invasively against the skin of the limb thereby impeding blood flow into and out of the limb". Also, the method of '131 does not, as the examiner states, impede outflow of blood from the limb. '131 describes the formation of a closed circuit created between the femoral artery and the femoral vein, washing blood away from the closed circuit, and recirculation of the virus through the circuit. Each of these steps inherently requires outflow of fluid from the limb at the same rate at which it is injected.

The examiner also states that the method of '131 inherently resulted in delivery and expression in skeletal muscle cells. Applicants respectfully disagree. '131 observed delivery

to BN175 or ROS-1 (osteo)sarcoma cells which were subcutaneously implanted into the right hindlimb of the experimental animals. '131 further states "No high uptake of IG.Ad.MLP.Luc by the liver or skeletal muscle of the isolated limb after ILP or intra-tumor injection."

Claim 39 has been rejected under 35 U.S.C. 102(a) as being anticipated by Von der Leyen. Applicants respectfully disagree. Von der Leyen did not inject polynucleotides into a vessel in a limb distal to a sphygmomanometer or obtain delivery of polynucleotides to skeletal muscle cells in a mammalian limb distal to a sphygmomanometer.

Von der Leyen did not, as the examiner contends, apply a sphygmomanometer to the skin of the limb. A sphygmomanometer has two components: an inflatable cuff and a pressure gauge. During its normal operation to measure blood pressure, the pressure exerted by the cuff is monitored using the pressure gauge. As indicated by Von der Leyen, they used only the pressure gauge of the sphygmomanometer to monitor the pressure of the transfection solution (see last sentence of page 2356). Further evidence that they did not apply a sphygmomanometer to a limb is: a) a sphygmomanometer will not fit on a rabbit limb, and b) a percutaneous translumenal coronary angioplasty manometer was substituted in the method for pressures higher than 300 mmHg (manometer: **1.** An instrument used for measuring the pressure of liquids and gases).

Von der Leyen also did not, as the examiner contends, implicitly teach obtaining delivery to skeletal muscle. First, the carotid artery was isolated (page 2356, second column, *Transfection procedure*) and therefore could not be surrounded by skeletal muscle. Second, the isolated vessel was surrounded by a clear polyethylene protective sheath. Polyethylene is water impermeable. Third, the artery was clamped at the proximal end of the sheath and ligated at the distal end of the sheath. Therefore, no solution injected in to this isolated section of artery could reach any skeletal muscle.

The examiner states that Von der Leyen observed delivery to skeletal muscle because DNA was forced through the blood vessel wall. Through the vessel wall, in this instance, refers only to layers within the vessel wall itself, the neointima, media and adventitia (see pg. 2360 col. 1). To determine the extent of delivery, Von der Leyen assayed luciferase and β-gal expression in vessel segments that were removed (harvested) from the rabbits (pg. 2357, col.

1, *Harvesting of vessels*). It would not be possible to observe delivery to skeletal muscle in a harvested section of blood vessel.

The examiner further contends that in the method of Von der Leyen, DNA may be diffused through the vessel wall during initial pressurization and through areas not covered by the sheath. However, during initial pressurization, the convective forces are the movement of the solution as it is being pumped into the isolated vessel to increase the pressure from zero mmHg to the final pressure. As stated above, the vessel was clamped at the proximal end and ligated at the distal end. Therefore there are not areas unprotected by the sheath. If there were, it would not be possible to create a pressurized segment of vessel with no convective forces.

Rejection of the claims under 35 U.S.C. 103:

The claims have been rejected as being unpatentable over Budker (1998) or Wolff et al. (US Patent 6,265,387) in view of Milas et al. (1997). The method of Milas does not result in delivery of nucleic acid to skeletal muscle as explicitly taught by Milas (page 2201, Col. 2). The examiner states, on page 11 of the action, that "Milas and Ye established the inability to obtain expression of proteins in muscle tissue using adenovirus and a clamped blood vessel." Therefore one would not have been motivated to apply the teaching of Milas with the teaching of Budker.

As Applicants have previously pointed out, brisk outflow of blood from the limb is critical to the method of Milas. The examiner states that the applicants' claims are not limited to impeding all blood flow into and out of the limb. Both claims 1 and 39 specifically state that blood flow to and from the limb is impeded.

Double Patenting:

The claims have been rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over Wolff et al. (US Patent 6,265,387) in view of Milas. As stated above, Milas did not teach impeding blood flow from the limb and explicitly taught that their method did not deliver nucleic acid to skeletal muscle.

The claims have been rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over Monahan et al. (US Patent 6,627,616) in view of Milas. As stated above, Milas did not teach impeding blood flow from the limb and explicitly taught that their method did not deliver nucleic acid to skeletal muscle. Therefore, there can not a been a reasonable expectation of success in applying the method of Milas with the method of '387.

The Examiner's objections and rejections are now believed to be overcome by this response to the Office Action. In view of Applicants' amendment and arguments, it is submitted that claims 1-3, 6, 7, 11, 12, 16-20, 24, 25, 28-31, 34-36, and 39-42 should be allowable. Applicants respectfully request a timely Notice of Allowance be issued in the case.

Respectfully submitted,

Mark K. Johnson Reg. No. 35,909
Mirus Bio Corporation
505 South Rosa Road
Madison, WI 53719
608-238-4400

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as express mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on this date: January 27, 2005 .

Kirk Ekena